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Effect of sodium hydroxide and salinity on yield and Properties of agar from *gracilaria dentata*

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ABSTRACT:
The effect of sodium hydroxide and salinity on the yield and gel strength of agar extracted from *Gracilaria dentata*, a red seaweed (agarophyte) was studied. The *G. dentata* were harvested manually from October 2010 to September 2011 from the coastal beaches of Shama, Biriwa and Kokrobite during low tides. The red seaweeds were dried and bleached in the sun and agar was extracted at 121^{o}C for 40 minutes. Pretreatment of *G. dentata* with concentrations of NaOH (0%, 3%, 6%, 9%, 12%) enhanced agar yield and gel strength. *G. dentata* treated with hot 0.1M Na_2CO_3 and 3% NaOH solutions, harvested from Shama gave the highest agar yield of 20.3% whilst that of Biriwa had the highest gel strength of 343.3gcm^{-2}. Salinity treatment and soaking time had a significant effect on agar yield and gel strength. *G. dentata* pretreated by soaking them in seawater ($5^{o}/_{oo}$,$15^{o}/_{oo}$,$25^{o}/_{oo}$,$35^{o}/_{oo}$) increased agar yield and but decreased the gel strength. Salinity values of $35^{o}/_{oo}$ and $5^{o}/_{oo}$ and soaking period of one hour gave the highest agar yield and gel strength of 58.7% and 73.3gcm^{-2} respectively.

Keywords: *Gracilaria dentata*, Agar, Seaweed, Sodium hydroxide, Salinity.

INTRODUCTION

Red seaweeds belong to a group of plants called algae. There are three main types of red seaweeds which occurred at the coastal beaches of Shama in the Western Region, Biriwa in the Central region and Kokrobite in the Greater Accra Region of Ghana. These were *Gracilaria dentata*, *Hypnea musciformis and Centroceras clavulatum*. The dominant red alga at these coastal beaches was *Gracilaria dentata* with a ground cover of 34–57%. The principal product derived from *Gracilaria spp*. is agar. About 50 % of the 25,000 to 30,000 metric tonnes of dry *Gracilaria spp*. used in agar production is harvested mainly from wild crops in the cool-temperate marine waters of Chile and Argentina [1] and [2]. The remainder comes from fishpond culture in Taiwan, Hainan Island, China and mainland China [3]. Minor amounts of dry *Gracilaria sp*. are harvested in Brazil and South Africa, but the data often are not included in the International Statistics. In addition, *Gracilaria spp*. as food, particularly as a table vegetable has become increasingly popular in the Caribbean Islands and Hawaii. Retail prices in these local markets are as high as US$6.00 for 6.5kg^{-1} of dry *Gracilaria spp*. in St. Lucia Island and up to US$3.50 for 5.00kg^{-1} of the wet seaweed in Hawaii [4].

Agar is a gelling hydrocolloid of commercial value, present in the cell walls of members of the Rhodophyta. It is insoluble in cold water, but soluble in hot water. A clear solution of 1.5 % will form a solid and elastic gel at a temperature of 32-39 °C and will not melt below 85 °C. Agar is used in countries like Japan, Australia and New Zealand to transport preserved cooked fish. The fish is protected from breaking by embedding it in a form of agar jelly. The agar also prevents the constituents of certain fishes such as herrings from blackening (detinning) the content of the can. The use of agar in food products is primarily associated with its high temperature tolerance. It is widely used in Europe and America as thickening agent in the manufacturing of ice creams, malted milks, jell candies and pastries. [5] reported that commercial processing using pretreatments like presoaking, grinding, alkali and or acid pretreatment modify the microstructure of the algae and chemical properties of the agar and thereby enhance agar yields. [6] also emphasized that pretreatment of *Gracilaria lemanaeformis* with cellulases before extraction enhanced agar gel strength and yields when the alga was not grinded. Micro-photographs of the algae presented by the group showed that cellulases produced microcracks in the external walls of the algal thallus and collapsed the internal cell walls. These structural changes apparently increased agar yield and gel strength by facilitating diffusion of the gelling polysaccharides.

As a result of an increasing demand of raw material for the extraction of agar, it has become a priority to identify new sources, and to improve the quality and yield of existing crops. No suitable substitute for agar has so far been synthesized and agar manufactured from members of the Rhodophyta will continue for many years to come. The present study is therefore aimed at finding out the combined effect of sodium hydroxide and salinity on the yield and gel strength of agar extracted from *Gracilaria dentata*.

MATERIALS AND METHODS

Sampling sites

Three representative study sites were selected for sampling. They were Shama in the Shama Ahanta East District of the Western Region; Biriwa in the Mfantsiman District of the Central Region and

Kokrobite in the Ga District of the Greater Accra Region of Ghana (Fig.1).

Collection of red seaweeds

Samples of *Gracilaria dentata* were randomly harvested manually from October 2010 to September 2011 at the coastal beaches of Shama, Biriwa and Kokrobite in their natural habitats in the intertidal zone during low tides. The collected samples were placed in large polythene bags and brought to the laboratory. The temperature, pH, salinity of seawater and light intensity were measured at the time of harvesting.

Identification of types of seaweeds

The types of seaweeds were identified using the key to their genera prepared by [7].

Treatment and sorting of red seaweeds

The harvested red seaweeds were thoroughly washed under running tap water for 20 minutes to remove salt crystals, soil and sand. The procedure was repeated five times and the red seaweeds manually separated from all other solid materials such as rock pieces, shells, calcareous inclusions and other seaweeds, remains of wood and plastics.

Bleaching and drying of red seaweeds

The fresh red seaweed samples were divided into two portions. One portion of the red seaweeds was put into hot water at 80-90 °C for one hour. This process helped to remove the red pigment (phycoerythrin) from the seaweeds. The second samples of red seaweeds were immersed into 0.1 M sodium carbonate (Na_2CO_3) solution at 80-90 °C for one hour. This process also helped to remove the red pigment (phycoerythrin) from the seaweed. The two samples of the red seaweeds were dried in the sun to bleach off the green pigment (chlorophyll). Sprinkling drying seaweeds with water in the evenings facilitated bleaching. The two samples were then dried to a constant weight and stored in polythene bags in a dry place.

Extraction of agar with aqueous sodium hydroxide (NaOH) solutions

Prior to agar extraction, 10 g of dried red seaweed samples were treated with 0 %, 3 %, 6%, 9 % and 12 % concentrations of NaOH solution at 90 °C for one hour in a water bath. The alkali solution was decanted and any alkali on the seaweed was neutralized with 0.1 M HCL and then washed in several changes of freshwater. Four hundred millilitres of distilled water was then added to the seaweeds in the conical flask and autoclaved for forty minutes at 121°C. The contents in the conical flasks were filtered while hot, through a strainer with very fine mesh size (0.315 mm) into a plastic container and the spare seaweed discarded. The hot filtrate was allowed to gel at room temperature after which it was frozen in the freezing chamber of a refrigerator at -8 °C for twenty-four hours. The frozen extract was thawed at room temperature and dried in an oven at 50°C to a constant weight which was recorded and percentage agar yield calculated. The agar was then shredded in a mill and stored. The procedure described was repeated for the second portion of the dried seaweed sample.

Extraction of agar at different salinity levels

Prior to agar extraction at different salinity levels, 10 g of dried seaweed samples were soaked for one hour in seawater with salinity levels of 5 ‰, 15‰, 25 ‰ and 35 ‰. After soaking, the agar was extracted following the method as already described above. The ratio of the dry seaweed material to seawater was 1: 40 (w/v). The dry extract obtained was weighed, grinded and stored.

The determination of the effect of salinity and alkali concentration on agar yield

The combined effect of salinity and alkali concentration on agar yield was determined when 10 g of dried seaweed samples in a conical flask were treated with 0 %, 3 %, 6 %, 9 % and 12 % NaOH solutions for one hour in a water bath at 90 °C. Washing, acid treatment and boiling of the samples followed the methods as described already for agar extraction but instead of distilled water, seawater of different salinities (5 ‰, 15 ‰, 25 ‰ and 35 ‰) were used for the extraction.

Determination of gel strength and grading of test agar samples

Gel strength was determined from 1.5 % hot agar solution prepared by heating 1.5 g of agar sample in 100 ml of distilled water and allowed to gel overnight in a plastic container measuring 5 cm by 5 cm and 2 cm in depth. 100 g, 200 g and 300 g standard weights, with base area of 1 cm^2 were successively placed on the gels for 20 seconds each time to observe which one of the weights will rupture the gel surface. The gel strength of 100 g/cm^2, 200 g/cm^2 and 300 g/cm^2 of agar surface are equivalent to grade 3, 2, and 1 agar respectively.

RESULTS

Types and distribution of red seaweeds

The types and distribution of red seaweeds at the sampling sites are presented in Table 1. Three main types of red seaweeds occurred at all the sampling sites. These were *Gracilaria dentata, Hypnea musciformis* and *Centroceras clavulatum (*Plate 1). The dominant red alga at all the three sampling sites in September 2010, was *Gracilaria dentata* with a ground cover of 34-57%, followed by *Hypnea musciformis* with 5 -15% ground cover and *Centroceras clavulatum with* 1-5% ground cover. The percentage ground cover for the three seaweeds was the same at all the three sampling sites (Table 1).

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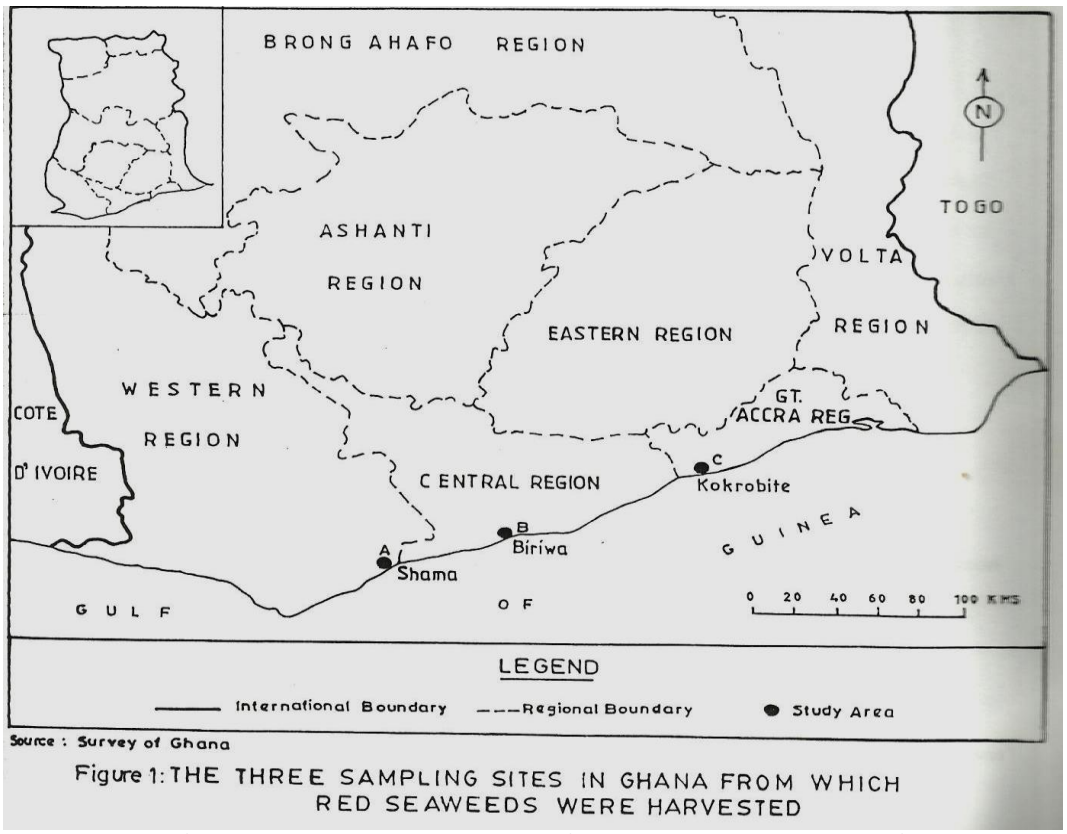

Fig 1: Shows the sampling sites of Shama in the Shama Ahanta East District of the Western Region; Biriwa in the Mfantsiman District of the Central Region and Kokrobite in the Ga District of the Greater Accra Region, where the study was conducted.

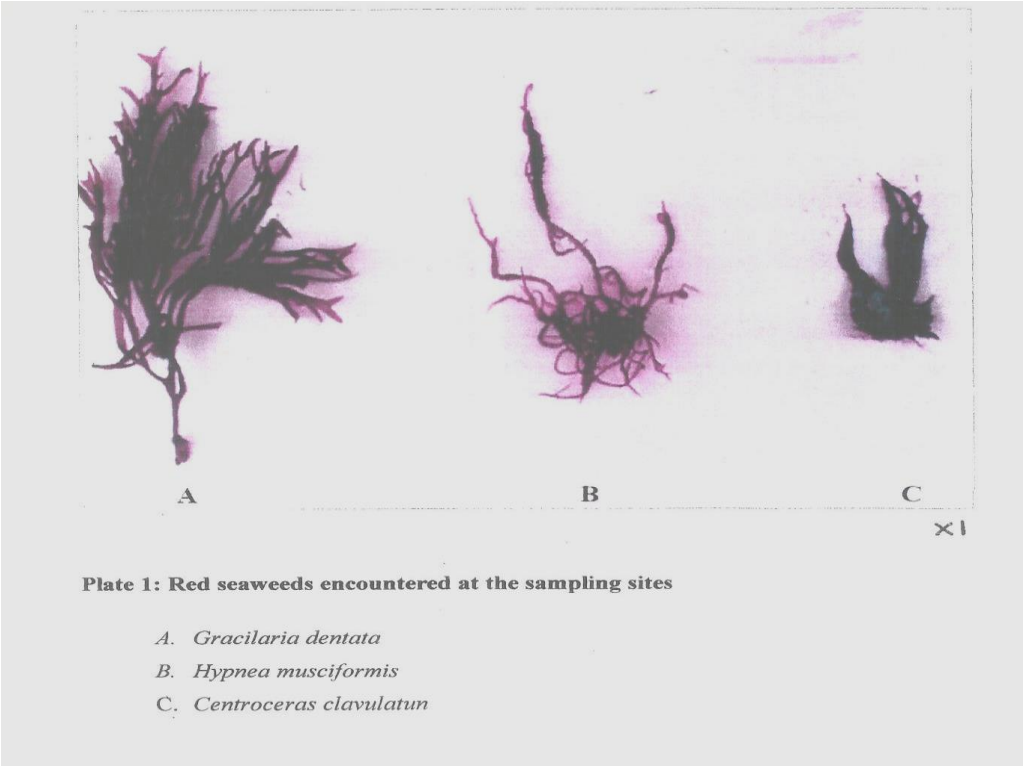

Fig 2: This shows plate 1 of the three main types of red seaweeds seen at all the sampling sites. They are *Gracilaria dentata*, Hypnea musciformis and Centroceras clavulatum.

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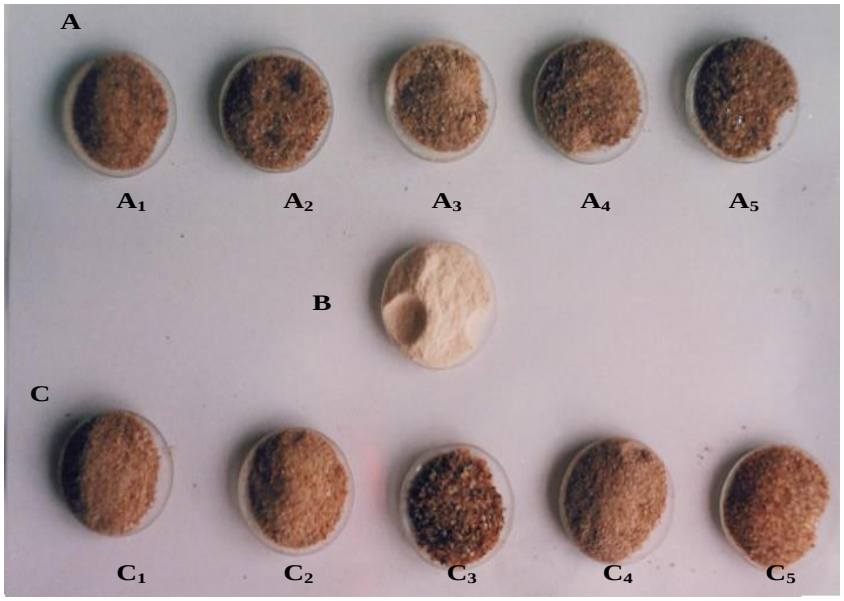


X ½

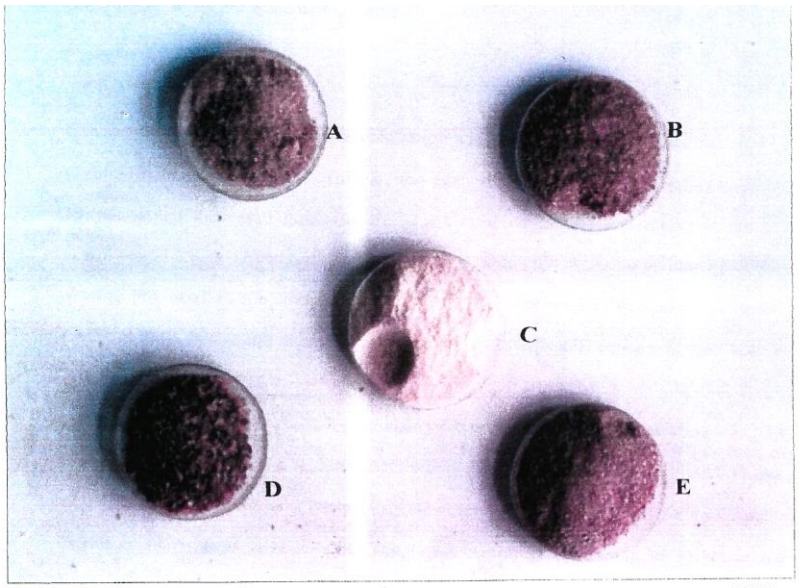
Plate 2: Dry powdered agar samples

A- Agar from agarophyte treated with hot Na_2CO_3 solution and the following concentration of NaOH solutions. A_1; 0.0% A_2; 3.0% A_3; 6.0% A_4; 9.0% A_5; 12.0%

B- Imported agar

C- Agar from agorophyte treated with hot water and the following concentration of NaOH solutions C_1; 0.0%, C_2; 3.0%, C_3; 6.0%, C_4; 9.0%, C_5; 12.0%

Fig 3: Shows plate 2 of the agar powder samples that were dried and treated with Na_2CO_3.



X ½

Plate 3: Dry powdered agar samples obtained from agarophyte treated with seawater with different salinities

A, $5^0/_{00}$, B; $15\ ^0/_{00}$, C; Imported agar D; $25^0/_{00}$ E; $35^0/_{00}$

Fig 4: Shows plate 3 of dry powdered agar samples obtained from agarophytes

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Table 1: Types and distribution of red seaweeds at sampling sites in September 2010

Types of red seaweeds	% ground cover at sampling sites.		
	Shama	Biriwa	Kokrobite
Gracilaria dentata	34-57	34-57	34-57
Hypnea musciformis	5-15	5-15	5-15
Centroceras clavulatum	1-5	1-5	1-5

Table 2: Mean percentage yield and gel strength of agar from *G. dentata* treated with hot water or hot Na_2CO_3 solution and extracted at different NaOH concentrations.

Sampling Site	NaOH Conc. (%)	Yield (%)		Gel strength (g/cm^2)	
		Hot water	Na_2CO_3 Solution	Hot water	Na_2CO_3 solution
Shama	0.0	15.6	17.6	263.3	253.3
	3.0	20.0	20.3	283.3	306.6
	6.0	19.0	18.3	266.6	266.6
	9.0	14.6	18.0	276.6	273.3
	12.0	17.0	16.0	273.3	283.3
	0.0	14.0	14.3	206.6	273.3
Biriwa	3.0	18.0	18.6	263.3	343.3
	6.0	15.0	13.6	256.6	303.3
	9.0	13.3	14.0	220.0	296.6
	12.0	11.6	12.3	233.3	273.3
	0.0	9.6	11.6	216.6	273.3
Kokrobite	3.0	15.3	18.3	293.3	310.0
	6.0	11.6	16.0	243.3	303.3
	9.0	10.3	14.0	216.6	266.6
	12.0	8.6	12.3	220.0	306.6

Table 3: Mean percentage yield and gel strength of agar from *G. dentata* extracted at different salinity values and soaking time.

Soaking time (hr)	Salinity ($^{o}/_{oo}$)	Yield (%)	Gel strength (g/cm^2)
1	5	27.6	73.3
	15	35.3	50.0
	25	36.3	46.6
	35	58.6	40.0
2	5	26.6	63.3
	15	36.3	43.3
	25	21.0	23.3
	35	48.6	10.0
3	5	21.3	56.6
	15	32.0	33.3
	25	35.3	10.0
	35	48.0	10.0
4	5	19.6	43.3
	15	24.6	23.3
	25	32.3	10.0
	35	41.0	10.0
24	5	19.3	36.6
	15	22.0	20.0
	25	31.0	10.0
	35	40.0	10.0

Table 4: Mean percentage yield and gel strength of agar from *G. dentata* treated with different salinity values and NaOH concentrations.

Salinity ($^{o}/_{oo}$)	NaOH Conc. (%)	Yield (%)	Gel strength (g/cm^2)
5	3.0	19.6	206.6
	6.0	17.6	153.3
	9.0	13.0	106.6
	12.0	21.3	240.0
15	3.0	21.0	150.0
	6.0	19.3	120.0
	9.0	18.6	86.6
	12.0	23.0	233.3
25	3.0	37.6	106.6
	6.0	21.6	40.0
	9.0	39.6	16.6
	12.0	39.6	133.3
35	3.0	38.6	10.0
	6.0	33.3	10.0
	9.0	32.6	10.0
	12.0	44.3	16.6

Effect of sodium hydroxide treatment on yield and gel strength of agar

The mean yield and gel strength of agar samples obtained from *G. dentata* treated with different concentrations of NaOH solution are presented in Table 2. Generally, the agar yield from Na_2CO_3 treated and untreated *G. dentata* initially increased to a peak and thereafter decreased gradually as the percentage NaOH concentrations increased (see Table 2).

At Shama, the agar yield from Na_2CO_3 treated *G. dentata* at 0.0% NaOH concentration (control) was 17.6%, which increased to 20.3% at 3.0% NaOH, decreasing to 16.0% at 12.0% NaOH concentration as shown in (Table 2 and Plate 2A). The untreated *G. dentata* produced a 15.6% agar yield at control, increasing to 20.0% at 3.0% NaOH and thereafter decreased gradually to 17.0% at 12.0% NaOH concentration (Table 2 and Plate 2C). But in contrast, the Na_2CO_3 treated *G. dentata* at Biriwa produced a 14.3% agar yield at control, increasing to 18.6% at 3.0% NaOH and then decreased gradually to 12.3% at 12.0% NaOH concentration (Table 2 and Plate 2A). For untreated *G. dentata*, the agar yield at control was 14.0%, which increased to 18.0% at 3.0% NaOH and thereafter decreased gradually to 11.6% at 12.0% NaOH concentration (Table 2 and Plate 2C).

Similarly, the Na_2CO_3 treated *G. dentata* at Kokrobite produced 11.6% agar yield at control, increasing to 18.3% at 3.0% NaOH and thereafter decreased gradually to 12.3% at 12.0% NaOH concentration (Table 2 and Plate2A). For untreated *G. dentata* the agar yield at control was 9.6%, which increased to 15.3% at 3.0% NaOH and then declined steadily to 8.6% at 12.0% NaOH concentration (Table 2 and Plate 2C).

Generally, the agar gel strength from Na_2CO_3 treated and untreated *G. dentata* initially increased to a peak and then decreased sharply and thereafter increased gradually as the percentage NaOH concentration increased (Table 2). At Shama, the agar gel strength from Na_2CO_3 treated *G. dentata* at control was 253.3 gcm^{-2}, which increased to 306.6 gcm^{-2} at 3.0% NaOH, decreasing to 273.3 gcm^{-2} at 9.0% NaOH and thereafter increased to 283.3 gcm^{-2} at 12.0% NaOH concentration (Table 2). The untreated *G. dentata* had agar gel strength of 263.3 gcm^{-2} at control, increasing to 283.3 gcm^{-2} at 3.0% NaOH and then decreased to 276.6 gcm^{-2} at 9.0% NaOH and thereafter decreased gradually to 273.3 gcm^{-2} at 12.0% NaOH concentration (Table 2).

At Biriwa, the Na_2CO_3 treated *G. dentata* had agar gel strength of 273.3 gcm^{-2} at control, which increased to 343.3 gcm^{-2} at 3.0% NaOH and then decreased sharply to 296.6 gcm^{-2} at 9.0% NaOH and thereafter declined gradually to 273.3 gcm^{-2} at 12.0% NaOH concentration (Table 2). For the untreated *G. dentata*, the gel strength at control was 206.6 gcm^{-2} which increased to 263.3 gcm^{-2} at 3.0% NaOH, decreasing to 220 gcm^{-2} at 9.0% NaOH and thereafter increased gradually to 233.3 gcm^{-2} at 12.0% NaOH concentration (Table 2). However, at Kokrobite the Na_2CO_3 treated *G. dentata* had a gel strength of 273.3 gcm^{-2} at control, which increased to 310.0 gcm^{-2} at 3.0% NaOH, decreasing to 266.6 gcm^{-2} at 9.0% NaOH and then increased sharply to 306.6 gcm^{-2} at 12.0% NaOH concentration (Table 2). For untreated *G. dentata*, the agar gel strength at control was 216.6 gcm^{-2}, increasing sharply to 293.3 gcm^{-2} at 3.0% NaOH and then decreased sharply again to 216.6 gcm^{-2} at 9.0% NaOH and thereafter increased gradually to 220.0 gcm^{-2} at 12.0% NaOH concentration (Table 2).

Effect of salinity treatment on yield and gel strength of agar samples

The mean yield and gel strength of agar obtained from *G. dentata* extracted in seawater with different salinities are presented in Table 3. Generally, an increase in salinity increased the percentage agar yield whilst an increase in soaking time resulted in a corresponding decrease in percentage agar yield (Table 3). The agar yield obtained from *G. dentata* soaked in seawater with salinity values of $5^0/_{00}$; $15^0/_{00}$; $25^0/_{00}$, and $35^0/_{00}$ for one hour was 27.6%; 35.5%; 36.3%, and 58.6% respectively (Table 3 and Plate 3). When the soaking time was doubled to two hours, the agar yield at salinity values of $5^0/_{00}$; $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ was 26.6%. 36.3%, 21.0% and 48.6% (Table 3 and Plate 3). Similarly three hours of soaking time yielded 21.3%; 32.0%; 35.3%, and 48.0% agars at salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ respectively (Table 3 and Plate 3). However when the soaking time was increased to four hours, the agar yield obtained at salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ was 19.6%, 24.6%, 32.3% and 41.0% respectively (Table 3 and Plate 3). At twenty-four hours of soaking time the agar yield at salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$ and $35^0/_{00}$ was 19.3%, 22.0%, 31.0% and 40% respectively (Table 3 and Plate 3).

In general increased salinity and soaking time resulted in a corresponding decreased in agar gel strength (Table 3). The agar gel strength obtained from *G. dentata* soaked in seawater with salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ for one hour was 73.3 gcm^{-2}, 50 gcm^{-2}, 46.6 gcm^{-2}, and 40.0 gcm^{-2} respectively (Table 3). When the soaking time was increased to two hours, the strength of the agar gel at salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ was 63.3 gcm^{-2}, 43.3 gcm^{-2}, 23.3 gcm^{-2} and 10.0 gcm^{-2} respectively (Table 3). Similarly the gel strength of agar obtained from *G. dentata* soaked in seawater with salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$, and $35^0/_{00}$ for three hours was 56.6 gcm^{-2},

33.3 gcm^{-2}, 10.0 gcm^{-2} and 10.0 gcm^{-2} (Table 3). At four hours of soaking time, the agar gel strength at salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$ and $35^0/_{00}$ was 43.3 gcm^{-2}, 23.3 gcm^{-2}, 10 gcm^{-2}, and 10 gcm^{-2} respectively (Table 3). However *G. dentata* soaked in seawater with salinity values of $5^0/_{00}$, $15^0/_{00}$, $25^0/_{00}$ and $35^0/_{00}$ for twenty-four hours had a gel strength of 36.6 gcm^{-2}, 20 gcm^{-2}, 10 gcm^{-2}, and 10 gcm^{-2} respectively (Table 3).

The combined effects of sodium hydroxide and salinity treatments on yield and gel strength of agar samples

The mean yield and gel strength of agar obtained from *G. dentata* treated with different concentrations of NaOH and extracted in seawater with differing salinities are presented in Table 4. Generally, an increase in NaOH concentration and salinity values increased the agar yield (Table 4). At salinity value of $5^0/_{00}$, the agar yield at 3.0% NaOH concentration was 19.6%, which decreased gradually to 17.6% at 6.0% NaOH and thereafter increased sharply to 21.3% at 12.0% NaOH concentration (Table 4). At salinity value of $15^0/_{00}$, *G. dentata* produced a 21% agar yield at 3.0% NaOH concentration, decreasing gradually to 19.3% at 6.0% NaOH and then increased to 23% at 12.0% NaOH concentration (Table 4). Similarly at salinity value of $25^0/_{00}$ the agar yield at 3.0% NaOH concentration was 37.6% which decreased to 21.6% at 6.0% NaOH and thereafter increased sharply to 39.6% at 12.0% NaOH concentration (Table 4). However, at salinity value of $35^0/_{00}$ G. dentata produced 38.6% agar yield at 3.0% NaOH concentration decreasing to 33.3% at 6.0% NaOH and then increased sharply to 44.3% at 12.0% NaOH concentration (Table 4). In general, an increase in NaOH concentration and salinity value decreased the agar gel strength (Table 4). At salinity value of $5^0/_{00}$, the agar gel strength at 3.0% NaOH concentration was 206.6 gcm^{-2}, which decreased to 153.3 gcm^{-2} at 6.0% NaOH and then increased to 240.0 gcm^{-2} at 12.0% NaOH concentration (Table 4).

Similarly at salinity value of $15^0/_{00}$, *G. dentata* produced a gel strength of 150 gcm^{-2} at 3.0% NaOH concentration, decreasing to 120 gcm^{-2} at 6.0% NaOH and thereafter increased sharply to 233.3 gcm^{-2} at 12.0% NaOH concentration (Table 4). At salinity value of $25^0/_{00}$, the gel strength at 3.0% NaOH concentration was 106.6 gcm^{-2} which decreased to 40.0 gcm^{-2} at 6.0% NaOH and then rose sharply to 133.3 gcm^{-2} at 12.0% NaOH concentration (Table 4). However at salinity value of $35^0/_{00}$, *G. dentata* produced a gel strength of 10.0 gcm^{-2} at 3.0% NaOH concentration which then remains steadily at both 6.0% and 9.0% NaOH concentrations and thereafter increased to 16.6 gcm^{-2} at 12.0% NaOH concentration (Table 4).

DISCUSSION

The establishment and growth of agar industry in Ghana will depend on the availability of red seaweeds. The studies revealed that red seaweeds abound in the coastal beaches of Shama, Biriwa and Kokrobite. Out of the three red seaweeds, *Gracilaria dentata, Hypnea musciformis, Centroceras clavulatum* identified at the sampling sites, *Gracilaria dentata* had the highest percentage ground cover at the three sampling sites (Table 1). Studies have also shown that the genus *Gracilaria (sensulato)* is currently the most important alga for the production of agar, and the basis of a multimillion-dollar industry, growing at a historical rate of 2% per year [8].

Pretreatment of *G. dentata* with NaOH was found to increase agar yields. This finding agrees with the observation by [5] who reported that pretreatment with NaOH modified the micro-structure of the algae to enhance agar yields. [6] also reported that NaOH disrupted the cell layers in the red algae to facilitate diffusion of agar out of the cells. The inverse relationship between sulphate content and gel strength has been well established [10, 11], which gives credence to the widespread commercial use of an alkali-modification of dried *Gracilaria* seaweed or its agar to improve its gel strength. NaOH concentrations had a highly significant effect on the gel strength [8] reported that various concentrations of aqueous NaOH, gave agars which varied significantly in gel strength. [11, 12] also showed that NaOH concentrations significantly increased agar gel strength. The NaOH solution hydrolyzed sulphate groups in the agar into anhydrous galactose to increase the gel strength of the agar [13]. It is well known that the gel properties of many *Gracilaria* agars can be improved by treatment with alkali [14-16] which converts L-galactose-6-sulphate to 3, 6 - anhydro-L-galactose.

Distinct changes in gel strength and agar yield coincided with the changes in environmental conditions. Results from this present study showed that salinity treatment had a high significant effect on agar yield and gel strength. Pretreatment of *G. dentata* with seawater with different salinities ($5^o/_{oo}$-$35^o/_{oo}$) increased agar yields but decreased the gel strength. However, this finding contrasts the results of [17]. He reported that agar content was typically greater in plants grown at $17^o/_{oo}$ than $33^o/_{oo}$ whilst lower salinities of $17^o/_{oo}$ led to reduction in agar gel strength than $33^o/_{oo}$. [18], also reported that salinities of $17^o/_{oo}$ consistently resulted in low productivity and low agar gel strength than either 25 $^o/_{oo}$ or $33^o/_{oo}$. Salinity dilution by rainfall or river run-offs into marine habitats might also affect agar quality. During the rainy season, salinity is low, thus energy is channeled less to osmoregulatory activities than to production of new cells and storage of food reserves thus resulting in a

decrease in gel strength during this period. Low gel strength may also be due to high sulphated polysaccharide content [19]. These highly sulphated polysaccharides, which do not gel, represent biological precursors of agarose and related polysaccharides, which are responsible for gel formation in agar [20, 21]. High salinity may also increase sulphate content as a response to changes in the environmental conditions. However, the differences in the results obtained might indicate that *Gracilaria* can have broad tolerances to changes in salinity [22].

The interactive effect of salinity and NaOH concentration greatly influenced agar yield and gel strength. This suggests that these two factors can influence the overall agar content. Plants treated with both 3% NaOH concentration and seawater with salinity value of $5^{o}/_{oo}$ produced the strongest gel. In contrast, [8] who grew *Gracilariopsis heteroclada* at four salinity levels and treated the plants with three different concentrations of aqueous NaOH, reported that plants grown at salinity value of $24^{o}/_{oo}$ and treated with 3% NaOH produced the strongest gel of 850 gcm^{-2}. The differences might be due to the response of different *Gracilaria* species to salinity treatment. The source of the raw material, type of alkali, its concentration and the pretreatment time play significant roles in obtaining high agar yield and gel strength.

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